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Effect of Atrial Natriuretic Peptide on Muscle Sympathetic Activity and Its Reflex Control in Human Heart Failure

Beth L. Abramson, MD; Shin-ichi Ando, MD, PhD; Catherine F. Notarius, PhD; Gerard A. Rongen, MD, PhD; John S. Floras, MD, DPhil

Background—The purpose of this study was to determine if atrial natriuretic peptide (ANP) exerts a relative inhibitory effect on muscle sympathetic nerve activity (MSNA) at rest and during nonhypotensive lower body negative pressure (LBNP) in heart failure, as in healthy subjects.

Methods and Results—Fifteen men (age 39 ± 2 years [mean ± SE]) with dilated cardiomyopathy (ejection fraction 18 ± 3%) received intravenous ANP (50 μg bolus, then 50 ng·kg⁻¹·min⁻¹) and nitroglycerin (NTG, 8 mg/min) as a hemodynamic control. During each infusion MSNA, blood pressure (BP), central venous pressure (CVP), and heart rate (HR) were recorded before and during LBNP at −6 and −12 mm Hg. NTG and ANP caused similar and significant reductions in CVP and diastolic BP, but resting MSNA did not increase with either infusion. LBNP at −6 mm Hg lowered CVP (P < 0.05), whereas LBNP at −12 mm Hg caused significant reductions in CVP, systolic BP, and diastolic BP. These effects of nonhypotensive and hypotensive LBNP on MSNA were lower both before and with LBNP during ANP (P < 0.02). Nonhypotensive LBNP increased MSNA during NTG (+133 ± 68 Units; P < 0.001) but not during ANP infusion (+24 ± 23 Units; P = NS).

Conclusions—These observations are consistent with the concept that ANP exerts a sympathoinhibitory action in heart failure. This is most evident in response to reductions in atrial pressures that do not affect systemic BP. (Circulation. 1999;99:1810-1815.)

Key Words: atrial natriuretic factor ■ blood pressure ■ heart failure ■ nervous system, autonomic ■ nitroglycerin

In addition to its natriuretic, diuretic, and vasorelaxant properties, atrial natriuretic peptide (ANP) can modulate the autonomic nervous system by sensitizing arterial and cardiac baroreceptor afferent nerve endings, inhibiting sympathetic ganglionic neurotransmission, and by a central neural action.¹⁻⁷ We have characterized, in healthy humans, a relative inhibitory effect of ANP on efferent sympathetic nerve traffic to calf muscle (muscle sympathetic nerve activity, MSNA).³ This action was evident under resting conditions and when cardiac mechanoreceptor discharge was first inhibited, then stimulated by the graded application and withdrawal of nonhypotensive lower body negative pressure (LBNP).³ These observations suggest a potential sympathomodulatory role for exogenous ANP or neutral endopeptidase inhibitors (which increase endogenous ANP) in conditions characterized by increased postganglionic MSNA, such as heart failure (HF).⁸⁻⁹

Survival in HF is inversely related to the degree of sympathoneural activation and in particular, noradrenergic drive to the diseased myocardium.¹⁰⁻¹¹ We have proposed that inhibition of sympathetic outflow to the heart and periphery should improve the prognosis of patients with HF.¹² Thus far, this hypothesis has not been tested directly in any large-scale mortality trial. Augmenting plasma ANP concentrations may be one such therapeutic intervention.

Plasma ANP concentrations rise in relation to the severity of HF.¹³ In its initial stages, this might serve to restrain sympathetic nervous system outflow to the kidney or skeletal muscle¹⁴ and slow disease progression. ANP could decrease sympathetic nerve traffic through one or more of the neural mechanisms described above or nonspecifically, as a consequence of its hemodynamic and diuretic actions. Pulmonary artery and left ventricular end-diastolic pressures are important hemodynamic correlates of sympathetic outflow to the heart and skeletal muscle in HF.⁸⁻¹⁵⁻¹⁶ The existence of these positive relations suggests that there may be an important sympathoexcitatory stimulus in HF arising from ordinarily quiescent afferent nerve endings situated in “low-pressure” chambers and vessels. Indeed, in patients with severe HF, Kaye et al.¹⁷ were able to inhibit norepinephrine (NE) spill-over across the heart by infusing sodium nitroprusside (SNP), which lowered pulmonary artery and cardiac filling pressures. Thus by reducing cardiac, pulmonary, and peripheral venous pressures and thereby deactivating these several excitatory stimuli, ANP might have greater sympathoinhibitory effects in patients with HF than in normal subjects.
Alternatively, the sympathoneural effects of ANP may be blunted. There is controversy as to whether the hemodynamic, natriuretic, and diuretic effects of endogenous or exogenous ANP are attenuated in HF.18–23 Previous investigators have used plasma NE concentrations as an indirect index of the effects of ANP on sympathetic activity in HF, with conflicting results.19–22 Because ANP infusion increases total body NE clearance,23 these observations cannot address the issue of whether ANP has sympathoinhibitory actions in human HF. In contrast, the microneurographic technique permits the direct recording of efferent sympathetic discharge to skeletal muscle. MSNA is increased in young subjects with dilated cardiomyopathy.9 The effect of ANP on MSNA in such patients has not been reported.

To test the hypothesis that ANP inhibits postganglionic MSNA in HF, we adapted the protocol used in our previous experiments in healthy subjects. We had 2 aims: (1) to determine the effect of exogenous ANP on hemodynamics and on efferent MSNA under resting conditions and (2) to determine whether ANP alters the cardiopulmonary or arterial baroreceptor reflex control of MSNA in these patients. We applied nonhypotensive and hypotensive LBNP for this purpose. As in our previous experiments, NTG was infused as a hemodynamic control to ensure that effects on MSNA were specific to ANP rather than a nonspecific response to reductions in cardiac filling pressures or to increases in stroke volume (SV).3,15

Methods

Subject Selection

Fifteen nonsmoking men with idiopathic dilated cardiomyopathy, 27 to 59 years of age (39±2 years, mean±SE), in sinus rhythm and stable condition, were recruited from our Heart Failure Service. They were characterized by normal coronary arteries, left ventricular end-diastolic dimensions ≥60 mm, and a mean ejection fraction, as assessed by radionuclide angiography, of 18%±3%. Patients were classified as New York Heart Association class I (n=1), II (n=6), III (n=7), or IV (n=1) and were prescribed 1 or more of angiotensin-converting enzyme (ACE) inhibitors or receptor antagonists (n=14), digitals (n=13), diuretics (n=10), and β-blockade with metoprolol (n=7). Subjects abstained from diuretics for 12 hours before their study. This protocol was approved by our University Human Subjects Review Committee. Informed written consent was obtained from all participants.

Procedures

Studies were conducted in the morning. Subjects emptied their bladder, then lay supine in a chamber built for recording MSNA from the right peroneal nerve during LBNP. BP was measured from the left arm by an automatic cuff recorder. A catheter was placed in a left forearm vein for infusions. A central venous catheter was placed in an antecubital vein of the right arm. A respiratory belt was secured around the abdomen. Central venous pressure (CVP) and respiratory excursions were recorded simultaneously with heart rate (HR), lead II of the ECG, and MSNA onto paper3 and stored on computer for analysis with the use of a LabVIEW-based program (National Instruments).24 MSNA was expressed as frequency (bursts/min), incidence (bursts/100 cardiac cycles), and integrated nerve activity (the product of burst frequency and mean burst amplitude, which is a quantitative representation of the strength of each burst).8,24,25 Because the application and release of LBNP might shift the microneurode and influence mean burst amplitude, or because in some subjects experiments might be performed in 2 sessions, we identified the maximum amplitude occurring during a 3-minute interval before each infusion and assigned this a value of 1000 U. All bursts recorded during that particular infusion were calibrated against this reference to derive normalized integrated sympathetic nerve activity (NISNA).3,25 SV was calculated over 10 or more consecutive cardiac cycles from 2-dimensional echocardiographic and continuous wave Doppler recordings.3,9

Plasma ANP concentrations were determined with a competitive enzyme immunoassay (Peninsula Laboratories Inc) with a detection limit of 20 pg/mL, <10% intra-assay variation, and 14% interassay variation. Blood was collected into prechilled tubes containing EDTA and aprotinin (500 KIU). After centrifugation, plasma samples were stored at –70°C. For analysis, thawed samples were acidified and extracted on Sep-Pak C18 columns (Waters Ltd).

Protocol

After instrumentation, subjects lay quietly for 10 to 15 minutes to establish baseline values. Before each infusion, baseline BP, MSNA, and CVP were determined over 3 minutes. The infusions were NTG (8 mg/min), followed by a 10- to 20-minute washout period, then ANP (50-µg bolus over 3 minutes followed by 50 ng · kg⁻¹ · min⁻¹). Blood was drawn 10 minutes into each infusion. After 20 minutes, the response to each infusion was assessed over a second pre-LBNP baseline period of 3 minutes. Infusions were continued and subjects were submitted to graded LBNP first at –6 mm Hg for 3 minutes, followed by –12 mm Hg for 8 minutes. Blood was again drawn during the last minute of LBNP. Negative pressure was withdrawn slowly to maintain a stable microneurographic site. Five minutes later, SV was measured, and cardiac output was derived from SV and HR. The infusion was then stopped. In 4 subjects, the protocol was conducted on 2 days because of MSNA site loss before the ANP infusion. There were no changes in medications or the clinical status of these patients between these 2 visits.

Statistical Analysis

Baseline values before each infusion and the specific effect of each infusion on baseline values were assessed by 2-way ANOVA. A 2-way ANOVA for repeated measures was applied to compare the effect of each infusion (NTG, ANP) on responses to each level of LBNP (0 mm Hg, –6 mm Hg, –12 mm Hg). Main effects were considered to be significant at the 95% confidence level, and a post hoc Student-Neuman-Keuls test was applied to assess differences between means. All data are reported as mean±SE.

Results

Effect of Infusions on Baseline Values

There was no significant difference in any baseline value for hemodynamics or MSNA before these 2 infusions (Table).

Hemodynamics

NTG lowered diastolic BP (DBP) (–9.1±1.5 mm Hg; P<0.0001) and CVP (–1.4±0.4 mm Hg; P=0.003) but did not affect systolic BP (SBP) (–3.7±2.1 mm Hg) or HR. ANP infusion, which increased its plasma concentration from 190±37 pg/mL to 1635±262 pg/mL (n=14, P<0.001), lowered DBP and CVP (–5.8±1.8 P=0.004, and –2.5±0.6 mm Hg P=0.0006) without affecting HR and lowered SBP (–4.7±2.1 mm Hg; P<0.03) (Table and Figure 1). There were no significant differences between the effects of NTG and ANP on SBP, DBP, CVP, HR, SV (51±7 vs 52±7 mL), or cardiac output (3.73±0.55 vs 3.92±0.53 L/min).

Sympathetic Activity

Both NTG and ANP caused significant reductions in mean values for both DBP and CVP without eliciting anticipated
reflex increases in MSNA.\textsuperscript{3,24–28} There was no difference in the effect of NTG and ANP on MSNA (Table).

There were significant but similar inverse relations between MSNA burst frequency at baseline and the response to these infusions (Figure 2). When the subgroup of 8 subjects with class III and IV heart failure (and the greatest degree of sympathetic activation) was considered separately, neither NTG (+0.2±2.1 bursts/min; SD 2.5±0.6) nor ANP (−3.5±1.9 bursts/min; SD 4.4±2.5 Units) affected MSNA significantly.

**Effect of Infusions on Responses to LBNP**

**Hemodynamics**

LBNP at −6 mm Hg caused significant reductions in CVP (P<0.05) without inducing systemic hypotension, whereas LBNP at −12 mm Hg caused significant reductions in CVP, SBP, and DBP (Table). The effects of nonhypotensive (−6 mm Hg) and hypotensive (−12 mm Hg) LBNP on SBP and DBP, CVP, and HR were similar during infusion of ANP and NTG. During the ANP infusion, 1 subject developed presyncope at the onset of LBNP. Subsequent paired comparisons were based on the remaining 14 subjects.

**Sympathetic Activity**

Although the hemodynamic stimuli to reflex sympathetic activation during LBNP were similar during both infusions, MSNA was significantly lower during ANP across all conditions (ie, both before and during application of LBNP) whether expressed as burst frequency (P<0.02) or burst incidence (P<0.02) (Figure 3). In response to nonhypotensive LBNP (−6 mm Hg), burst frequency (+3.2±2.2 bursts/min; SD 6.1±1.5) and burst incidence (+4.1±2.5 bursts/100 cardiac cycles; P<0.05) increased reflexively during NTG infusion but not during the ANP infusion (+0.6±1.2 bursts/min and +0.4±1.2 bursts/100 cardiac cycles, respectively). Normalized integrated sympathetic nerve activity increased by 133±68 Units (P<0.001) during NTG infusion but not...
during ANP (+24±23 Units, P=NS). In response to hypotensive LBNP, normalized integrated sympathetic nerve activity increased significantly during both infusions (+247±120 Units for NTG, P<0.001; +177±69 Units for ANP, P<0.005) (Table, and Figure 4).

Discussion
Most investigators have focused on the hemodynamic, renal, and endocrine actions of ANP in HF, whereas we have directed our attention at interactions between this peptide and the autonomic nervous system. Our previous experiments in young men with normal ventricular function demonstrated a relative inhibitory effect of ANP on MSNA at rest and during nonhypotensive LBNP.3,6 Our present objective was to determine whether ANP exerts a similar action in young patients with HF and increased central sympathetic outflow caused by dilated cardiomyopathy, and if so, to determine whether ANP alters reflex sympathoneural responses to reductions in atrial or systemic arterial pressure. NTG was infused as a control to ensure that any effect on MSNA was due to a specific neural action of ANP rather than a nonspecific reflex response to any hemodynamic changes caused by these infusions.3

In subjects with normal ventricular function, reductions in either DBP or CVP increase MSNA reflexively by decreasing the firing rate of afferent nerves arising from arterial24,26 and cardiopulmonary3,27 mechanoreceptors, respectively. Reductions in diastolic and atrial pressures caused by SNP infusion increase total body NE spillover by the same extent in subjects with normal ventricular function and HF.29 In the present series, ANP infusion lowered both DBP and CVP, yet MSNA did not increase in response to these hemodynamic changes, an observation consistent with a sympathoinhibitory action of this peptide. However, NTG also lowered DBP and CVP without increasing MSNA reflexively. By contrast, in healthy subjects, there was a distinct and significant difference in the MSNA response to ANP when compared with SNP.3 The absence of a reflex increase in MSNA during the first part of this study is therefore consistent with a sympathoinhibitory effect of both vasodilators in heart failure rather than a specific neural action of ANP.

Two nonspecific inhibitory mechanisms could account for the similar MSNA responses to ANP and NTG. There is a strong positive relation in patients with HF between sympathetic activity and both pulmonary artery and capillary wedge
pressures. Consequently, reductions in cardiac filling pressures caused by either infusion might diminish these stimuli to central sympathetic outflow. Second, reductions in right atrial pressure will release pericardial constraint on left ventricular filling and as a consequence increase left ventricular SV and stimulate inotropically sensitive left ventricular mechanoreceptors. SV was similar during these 2 infusions.

By studying only treated patients, we may have underestimated any sympathoinhibitory effect of ANP in severe HF. However, ANP infusion did not lower MSNA significantly in the subgroup with class III and IV HF or in the class IV patient intolerant of ACE inhibition. It is more likely that any sympathoinhibitory effect of ANP, exerted by modulation of the cardiopulmonary baroreflex, was offset by the reflex response of arterial baroreceptors to the fall in DBP, which is the proximate stimulus to MSNA. Our group has compared total body and transcardiac NE spillover responses to ANP (1 μg/kg bolus then 100 ng/kg per minute) and NTG in HF. Both infusions caused similar reductions in cardiac filling pressures, but only ANP lowered DBP and at the same time increased total body NE spillover. There was a significant inverse relation between changes in BP and changes in NE spillover across the heart. These observations are consistent with the concept that the neural response to these vasodilators in HF is a function of the relative interaction between the sympathoexcitatory response to arterial baroreceptor unloading and a sympathoinhibitory response to reductions in cardiac filling pressures. A nonhypotensive stimulus may be required to demonstrate any inhibitory effect of ANP on cardiac and systemic sympathetic outflow in HF.

We therefore applied nonhypotensive LBNP. In our previous study in normal subjects, this intervention increased NISNA (by 80%) during NTG but had no effect on NISNA when ANP was infused. In the present series, the effects of nonhypotensive (−6 mm Hg) and hypotensive (−12 mm Hg) LBNP on SBP, DBP, CVP, and HR were similar during the 2 infusions. Despite these comparable hemodynamic stimuli to sympathetic activation, the reflex response, MSNA was significantly lower during ANP than during NTG both before and during the application of LBNP. The principal difference again emerged during nonhypotensive LBNP: MSNA increased by 28% during the NTG infusion (P<0.001) but by only 5% during the ANP infusion (P=NS). When hypotensive LBNP was applied there were significant increases in NISNA during both infusions. By comparison, in another study in patients with HF treated with ACE inhibitors, nonhypotensive LBNP alone increased MSNA by 26%. These findings are consistent with the concept that ANP exerts a specific sympathoinhibitory action in HF in response to a selective reduction in cardiac filling pressure. This would reduce the stimulus to firing of stretch-sensitive atrial and ventricular baroreceptor afferent nerves. Indeed, initial experiments in rats suggested that the hypotensive response to atrial peptides was augmented by withdrawal of sympathetic outflow, effected reflexively through sensitization of cardiac receptors with vagal afferents. Sensitization of cardiac mechanoreceptors could account for the present observations, whereas if 1 or more other potential sympathoinhibitory mechanisms, such as a reduction in sympathetic ganglionic neurotransmission, sensitization of aortic arch baroreceptors, or a brain stem action, were active, a difference in MSNA between ANP and NTG should have emerged under resting conditions.

Observations during the NTG infusion merit comment. In normal volunteers, we documented a 32% increase in MSNA in response to intravenous NTG. Recently, Noll et al reported that oral isosorbide dinitrate increased MSNA in healthy subjects, whereas oral captopril did not, and suggested that nitrate-induced sympathoexcitation may have adverse implications for treated patients with HF. However, their observations were likely due to the different effect of these 2 interventions on cardiac filling pressures, which were not reported. There was an inverse relation between MSNA at rest and the response to NTG in these patients with HF (Figure 2), with no net sympathoexcitatory action (Figure 1).

The present observations are consistent with 2 concepts. In patients with HF, in the setting of ACE inhibition, (1) both ANP and NTG lower CVP and DBP without eliciting reflex increases in MSNA. This nonspecific sympathoinhibitory effect may be a response to reductions in cardiac filling pressures; and (2) ANP exerts, in addition, a specific sympathoinhibitory action. This is most evident when atrial pressure is lowered by nonhypotensive LBNP, a maneuver that simulates upright posture. These findings suggest a potential sympathomodulatory role for endogenous ANP in mild to moderate HF and a potential therapeutic role for exogenous ANP or for neutral endopeptidase inhibition. However, when compared with responses in healthy subjects, this effect is modest, suggesting that patients with HF may be relatively resistant to the sympathoinhibitory actions of ANP. Loss of this restraint could contribute to sympathetic nervous system activation in HF.

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